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Original Article

Effect of the polyherbal cream of *Andrographis paniculata* and *Vernonia amygdalina* in Truama wound

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ABSTRACT

To evaluate the wound healing benefits of *Andrographis paniculata* (AP) and *Vernonia amygdalina* (VA) as a poly-herbal cream, using shear butter as the oil phase. Excision wound was inflicted on Wistar albino rats which were divided into groups of six rats each: Group 1 received no treatment while groups 2, 3, 4, and 5 were treated with the shear butter cream base (Sbc), AP, VA, AP+VA and SSD (Silversulphadiazine) respectively. Also antibacterial activities of the extracts AP, VA and AP + VA against typed *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Echerichia coli* were determined. Wound healing on days 3, 6, 9 and 12 showed significant ($P< 0.05$) improvement in wound closure in the group treated with AP+VA as compared to the other groups. The healing of rats treated with AP+VA cream was higher than those treated with SSD when assessed in terms of wound closure, epithelization and fresh hair growth. AP+VA also showed remarkable antibacterial activity against the three screened pathogens. The study showed enhanced wound healing for the polyherbal cream: AP+VA, in comparison to AP and VA as well as SSD.

Keywords: Wounds, Healing, *Androphis paniculata*, *Vernonia amygdalina*, formulated cream, antibacterial activity

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INTRODUCTION

Wounds are the physical injuries that result in opening or breaking of the skin. There are several broad classifications of wounds and may include open and closed wounds, as well as acute and chronic wounds [1,2]. Wounds constitute among the major causes of visits to hospitals worldwide: accounting for up to 30–42% of hospital attendance and 9% of deaths every year [3,4]. The healing of wounds is a complex cascade of events involving several cellular and biochemical processes [5,6]. The processes comprise of four tightly regulated overlapping phases: haemostasis, inflammation, proliferation or re-epithelialisation and remodeling. These corresponds to the restoration of the barrier function of the skin, destruction and removal of any invading pathogens and foreign debris, restoration of the local vasculature and tissues and, finally, remodelling of the wound site to resemble the intact uninjured tissue such that at the end of the process up to 80% original tensile strength of the original skin is restored [7]. Healing agents ultimately accelerates these processes.

Herbs and herbal materials constitute the major mainstay of various traditional medicines and traditional approach to wound care [8]. The holistic nature of most traditional medicine makes them a sensitive patient-centered healthcare system. The primary goal of traditional approach to wound management is to enhance patients' comfort and accelerate wound healing. In Africa, most traditional medicine practitioners manage wounds with herbal medicine. Many of the herbal products used for treating wound have a myriad of activities which include tissue regeneration, immune-boosting, anti-anxiety, antibacterial, anti-inflammatory, analgesic properties etc [9,10]. The products may be administered orally or applied directly on the wounds [8].

Many herbs with wound healing potential have been identified and authenticated by scientific methods. However only a few have been developed or formulated into products of conventional dosage form for therapeutic uses. *Andrographis paniculata* and *Vernonia amygdalina* are among herbs of known wound healing potential.

A, paniculata is an herbaceous plant that is popularly known as *King of bitters* and belonging to the family Acanthaceae [10,11]. It is a medicinal plant with a broad spectrum of uses. It has proven to be highly effective in its various conventional and folkloric indications [12,13]. *Andrographis paniculata* has been used for the treatment of many diseases including cancer, diabetes, hypertension, common cold, fever, leprosy, gonorrhea, scabies, boils, skin eruptions, ulcers and worm infections [14,15]. Its wound healing potential has also been established [11]. Biochemical analysis of the methanol extract showed that the extract was non-toxic when applied topically and no interference with normal body function [13].

Vernonia amygdalina, popularly known as bitter leaf, is an under shrub of variable height with petiolate green leaves of about 6 mm diameter, belonging to the family Asteraceae or Compositae [16, 17]. *V. amagdalina* is a tropical plant: the aerial parts that is commonly used is relatively non-toxic and safe for consumption and possesses several health benefits as well as a great potential as pharmaceutical leads for the treatment of certain diseases [16,18]. In Nigeria the leaf is a popular vegetable used in native soups and cousines as well as in several ethnomedicinal recipes [19,20]. The leaf and other part of plant have been used for the treatment of several health challenges such as fever, stomach disorder, jaundice, worm infestation, constipation, malaria, hiccups, kidney problems, amoebic dysentery, schistosomiasis, cough, wounds, diabetes, laxative, venereal diseases as well as bacterial and protozoal infections [21-23].

Many herbal medicine used in folk medicines are composed of complex recipes that contain more than one herb or herbal material. An important attribute of these native poly-herbal products include: synergism, toxicity buffering effect, multiple effects, reduction of side effect due dose reduction. *A. paniculata* and *V. amagdalina* have each been established to have wound healing activity however no work on the possible synergism or formulation into conventional dosage form for possible therapeutic consideration. This study seeks to evaluate the wound healing benefits of combining *A. paniculata* and *V. amagdalina* as a poly-herbal recipe formulated as a cream by using shear butter as the oil phase.

MATERIALS AND METHODS

The leaves of *Andrographis paniculata* and *Vernonia amagdalina* were collected in National Institute for Pharmaceutical Research and Development, (NIPRD), Idu, Abuja, Nigeria. The plants were identified and voucher specimens (NIPRD/H/6422/H6502) were deposited at the herbarium of NIPRD for reference. Methanol and Soft paraffin (BDH, Poole England). Silver sulphadiazine (Dermazine®), glycerol and sorbitan monooleate (Span80) were purchased from Sigma, Germany, *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeroginosa* (ATCC 27853), and *Escherichia coli* (ATCC25922) were purchased from Sigma, Germany. All other solvents and chemicals used were of analytical grade.

Preparation of the plant materials: The leaves of *V. amagdalina* and whole aerial parts of *A. paniculata* were washed and dried under shade for days. The dried plants were ground to coarse powder with the help of grinding mill and stored for further study.

Preparation of extracts: The leaves powder of *A. paniculata* (100 g) and *V. amagdalina* (100 g) were respectively extracted with 950 ml of methanol in soxhlet extractor for 48 hrs [9,24]. The methanol extract was concentrated under the vacuum in rotary flash evaporator and successively in hot air oven set to 60 ^{o}C for 48 hrs to obtain a solid mass. The extraction was repeated for four portions of the materials so as to obtain enough quantities of extract to carry out the studies. The dried extracts of each herb were pulled together and respectively stored in a wide mouthed plastic jar in a desiccators containing an active desiccant at room temperature (≈ 27 ^{o}C) until used.

Formulation of herbal cream: A hundred gram quantity of each cream type containing an equivalent 60 g of shear butter were prepared according to the formula presented in Table 1 and evaluated for their wound healing properties. An appropriate weight of the shear butter was transferred into an appropriately sized wide mouthed screw capped plastic jars which was placed in a water bath maintained at 70 ^{o}C. Sorbitan sesquioleate was then added to the melted shear butter. The contents of the plastic jars were respectively mixed with an electronic stirrer (REMI Motor Type RQ 122, REMI Electrotechnik Ltd, India) rotating at about 1500 rpm. The polysorbate 80 was mixed with the water and was gradually transferred into the jar containing the melted shear butter and surfactant until half the quantity has been introduced. The predetermined amounts of the herbal extract(s) were then respectively incorporated with continuous stirring. The drug was then transferred into the jar containing the shear butter with continuous stirring for about five minutes after which the remaining portion of the water and polysorbate 80 mixture were added with continuous stirring for another ten minutes [25].

Table 1; Formula for shear butter cream base, *A. paniculata,* V. *amydalina and A. paniculata + V. amydalina* extracts creams

Ingredients	Sample 1 (VA+AP)	Sample 2 (VA)	Sample 3 (AP)	Sample 5 (Cream base)
Shear butter (g)	60	60	60	60
VA (g)	3	6	-	-
AP (g)	3	-	6	-
Water (ml)	23.50	23.50	23.50	29.50
Polysorbate 80 (g)	4.26	4.26	4.26	4.26
Sorbitansesquioleate (g)	6.24	6.24	6.24	6.24

Characterization of Creams

Organoleptic evaluation: Sensory tests were adopted to characterize the creams by evaluating their appearance, texture and odour. These were also used to evaluate their stability, principally by monitoring their physical characteristic at different temperatures as well as evidence of creaming, coalescence and liquefaction when stored at 5 ±0.1°C, 28 ±0.1 and 40 ±0.1.

A 50 g quantities of each cream sample was stored in the refrigerator (≈5 ^{o}C), room temperature corresponding to 28 ^{o}C and in an oven set to 40 ^{o}C respectively. The sensory tests were carried out by observing the appearance, texture and odour every 7 days for 28 days [25].

Determination of pH: The pH of the freshly prepared creams was determined using the pH meter (Accumet Research AR10, Singapore) by immersing the probe of the pH meter into the creams. The pH of samples stored at the different temperatures: 5 ±0.1°C (in refrigerator), 28 ±0.1 and 40 ±0.1 respectively. Before determination the samples were allowed to stand for 1 h at normal laboratory condition to ensure equilibration. The pH of the creams was determined on day 1 after the creams had set and subsequently every 7 days for 28 days, then every 30 days up to 90 days [25, 26]. After each determination the samples were respectively restored to their analytical storage conditions.

Pharmacological evaluation

Animal selection and pretreatment: Fifty Wister albino rats of males and females sexes weighing about 190-200 g were obtained from the Animal House Unit of the Department of Pharmacology and Therapeutics, Bingham University Jos, Nigeria. The animals were maintained on pelleted feed, water (ad libitum), optimum light and temperature in accordance with the principles and guidelines of handling laboratory animals by the Nigerian Institutes of Health guide for the care and use of laboratory animals; Public No 85-23, revised 1985. The animal study was carried out after prior approval of the animal Ethical Committee of Bingham University, Jos, Plateau State Nigeria.

Skin irritation test: Five albino Wistar rats were chosen and caged individually with food and water given *ad libitum*. A 4 cm^2 area of dorsal portion of the rats were shaved with a shaving stick (BiC®) and wiped with methylated spirit. A 2 g quantity of the cream was applied over the site. The test sites were observed every 6 h for erythema and edema for a total duration of 48 h after application of the formulations in accordance to the earlier researcher [27].

Wound healing studies: The animals were divided into 5 groups of 6 animals each irrespective of sex after which the side of their central trunk was shaved (4 cm diameter) with a shaver and sterilized with ethanol (70%). Full skin thickness was then excised with size 2 sterile surgical blade to get a wound area measuring about 4 cm^2 having anaesthetized the animals with light chloroform [28,29]. Wounds were cleaned with normal saline animals placed in individual labeled cages, grouped and treated with various test preparations and standard drug as shown in Table 2.

The animals had their wounds treated daily for 21 days except for those in Group I (control). Wound area was carefully measured using a divider and thereafter estimated on a 30 cm rule. Progress of wound healing as characterized by epitheliazation, complete closure and hair regeneration were also monitored and documented by photo shots taken every 3 days.

Tables 2: Animal groupings for the wound healing studies

Groups	Test	Treatment
1	Control	No
2	Cream base	Yes
3	AP cream	Yes
4	VA cream	Yes
5	AP+VA	Yes
6	SSD	Yes

Antimicrobial Assay: The cream base and formulated cream of *A. paniculata* (AP), *V. amygdalina* (VA), *A. paniculata* + *V. amygdalina* (AP+VA), *were* tested for antimicrobial activity by disc diffusion sensitivity test method against three micro-organisms. They are *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escheria coli* (ATCC 25922). The colonies were standardize with 0.5 McFarland's standards and were grown in different Petri dish. SSD was used as the positive (standard) control. In each Petri dish was placed the sensitivity disc impregnated with the creams, extracts and the positive control. After incubation at 37 ^{0}C for 24 hours, the inhibition zones around the disc were measured. The result of antimicrobial activity test was reported as the diameters of the inhibition zones surrounding the discs measured in millimeter (mm). The experiment was done in triplicate [30].

Statistical analysis: Results were expressed as mean ±SEM. One- way ANOVA and Student t-tests were performed on data set. Differences were considered significant for P values <0.05.

RESULTS

Preparation of extracts: A yield of 21.5±1 % of dark green solid mass of *V. amagdalina* and 18.3±1% of lighter green dry solid of *A. paniculata* were obtained from the extraction process.

Formulation of cream and characterization

Cream Formulation: Elegant oil in water emulsion system cream of shear butter and herbal extracts were formed. Mixtures of ploysorbate 80 and sorbitan sesquioleate corresponding to Hydrophyle Lypophyle Balance (HLB) values of 15 and 3.7 respectively were blended in gravimetric ratio of 1: 4 to produce a system of HLB of value 10.7 that corresponds to an oil in water milieu.

Appearance: The freshly prepared creams of the *V. amygdalina*, *A. paniculata* and the combination of *V. amygdalina* and *A. paniculata* were green semi solid mass while the placebo shear butter cream is light cream coloured. There were no changes in the colours of the creams even after the 28 days assessment period. The creams stored at 5 ^{o}C and 27 ^{o}C did not show any change in colour. The herbal creams showed a tint of brownish green after 14 days of storage at 40 ^{o}C. All the creams stored at 5 ^{o}C appeared solidified throughout the 28 days period, while those stored at room temperature (≈ 27 ^{o}C) were a soft mass of cream. However those stored at 40 ^{o}C were a free flowing viscous mass.

The cream texture: The freshly prepared herbal and placebo shear butter creams when rubbed between the fingers produced similar texture characteristics that were smooth cooling feel. When stored at 5 ^{o}C (refrigeration) was a bit difficult to collect with the finger and disperse however after dispersion it showed the same feel. However at 40 ^{o}C the creams were easy to collect and the rubbing produced a lower viscosity pull between the fingers. There was no change in this texture at the end of the 4 week of assessment.

Liquefaction: All the freshly prepared creams of the herbal extracts and shear butter placebo cream on setting formed a soft creamy mass at 27 ^{o}C. When stored at 5 ^{o}C the creams formed a solid mass. When stored at 40 ^{o}C, all the creams became fluid with the denser mass containing the extract

sedimenting at the bottom, however on shaking the emulsion reconstituted and reversed to its initial form.

pH of cream: The pHs of the various cream samples are presented in Table 3. The pHs of the shear butter base cream, VA, AP and VA+AP creams all showed similar pHs. There was no remarkable change in pH for the cream samples when stored in the different temperatures (5 ^{o}C, 27 ^{o}C and 40 ^{o}C) up to the 90 days period evaluated.

Table 3: Some physico-chemical and pharmacological properties of the formulated creams

No	Formulation	Appearance	pH	Texture	Consistency	Skin irritation test
1	Cream base	Light cream	4.8	Smooth	Creamy	No Erythema / Edema
2	AP	Green	5.0	Smooth	Creamy	No Erythema / Edema
3	VA	Green	5.0	Smooth	Creamy	No Erythema / Edema
4	AP+VA	Green	5.0	Smooth	Creamy	No Erythema / Edema

Pharmacological evaluation

Skin irritation test: No erytherma or edema was formed on the hair shaven skin of the rats when each of the cream sample was applied liberally.

The results of some critical physico-chemical and pharmacological evaluations of the formulated creams are represented in Table 3. An elegant oil in water emulsion cream of the base and herbal extracts were formed. This is oil in water milieu. The freshly prepared creams of the *V. amagdalina*, *A. paniculata* and the combination of *V. amagdalina* and *A. paniculata* were green semi solid mass while the placebo shear butter cream is light cream coloured. There were no changes in the colours of the creams even after the 28 days assessment period. The creams stored at 5 ^{o}C and 27 ^{o}C did not show any change in colour. The herbal creams showed a tint of brownish green after 14 days of storage at 40 ^{o}C. All the creams stored at 5 ^{o}C appeared solidified for the throughout the 28 days period, while those stored at room temperature (≈ 27 ^{o}C) were a soft mass of cream. However those stored at 40 ^{o}C were a free flowing viscous mass. The pH of all creams ranged from 4.8 to 5.0 throughout the study period. The freshly prepared herbal and placebo shear butter creams when rubbed between the fingers produced similar texture characteristics that were smooth cooling feel. When stored at 5 ^{o}C (refrigeration) was a bit difficult to collect with the finger and disperse however after dispersion it showed the same feel. However at 40 ^{o}C the creams were easy to collect and the rubbing produced a lower viscosity pull between the fingers. There was no change in this texture at the end of the 4 week of assessment. All the freshly prepared creams of the herbal extracts and shear butter placebo cream on setting formed a soft creamy mass at 27 ^{o}C. When stored at 5 ^{o}C the creams formed a solid mast. When stored at 40 ^{o}C, all the creams became fluid with the denser mass containing the extract flocculated at the bottom, however on shaking the emulsion reconstituted and reversed to its initial form. No erytherma or edema was formed on the hair shaven skin of the rats when each of the cream samples was applied liberally.

Wound healing studies: Fig. 1, indicates the rate of wound healing in the treated animals. There was increase in percentage of wound healing in all the groups of rats during the 12 days. From the third to twelfth day of treatment, the animals treated with AP+VA showed significantly greater healing effect than other groups ($P < 0.05$). AP+VA and SSD showed 100% wound healing property on the 12th day. The wound epithelizations at different days in the experimental rats are shown in Fig. 2. There was a significant enhance epithelization time in all the treated animals compared to controls during the 12 days ($P< 0.05$). AP+VA showed significantly greater wound healing time than other groups ($P <0.05$). On 12th day, complete wound healing ($0.00mm^{2)}$ was observed with AP+VA and SSD against ($210\ mm^2 \pm 0.26$) in controls.

Antimicrobial Assay: The antimicrobial activities of the creams are shown in Fig. 3. The standard drug SSD showed highest degree of inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. This was followed by inhibitory effect of AP+VA on the three tested bacteria. The AP showed moderate bacterial activity than the VA. However the cream base had no inhibitory effect on *Pseudomonas aeruginosa* and *Escherichia coli*.

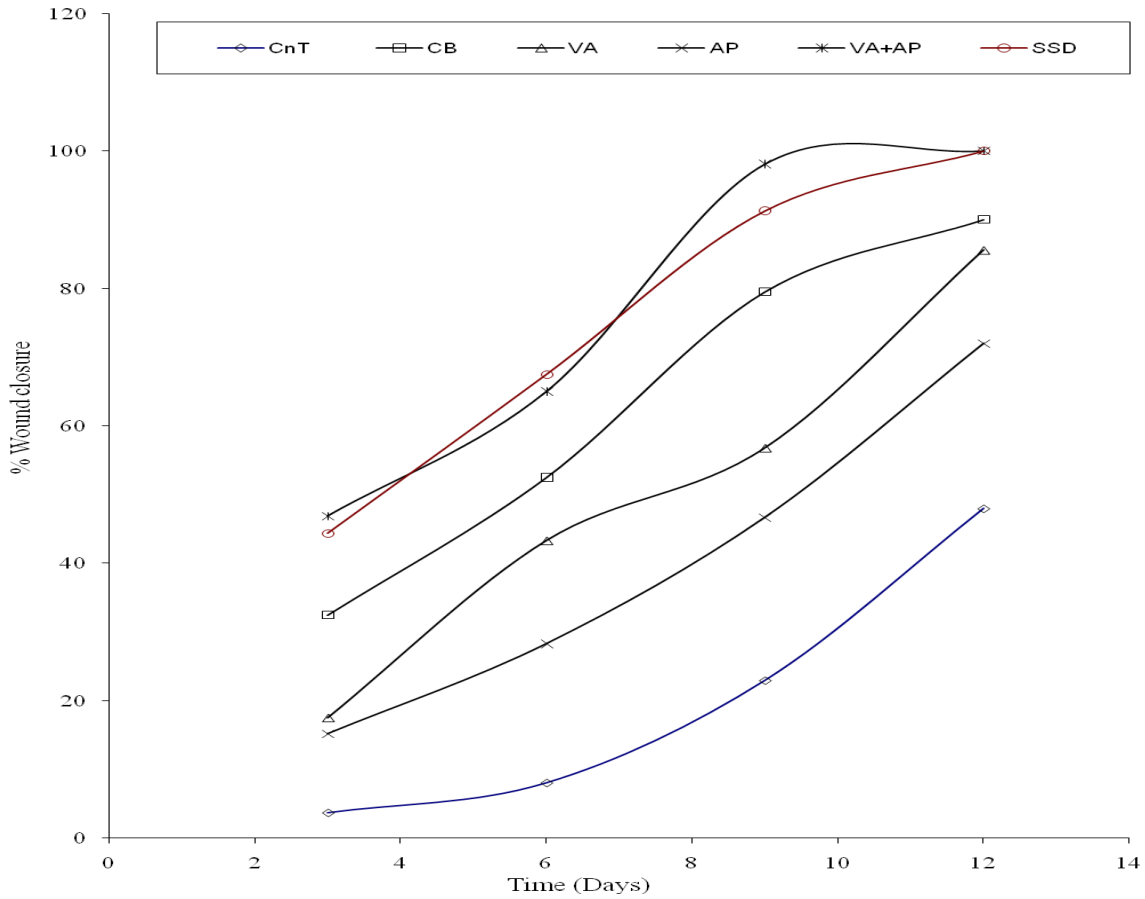

Fig. 1: Percentage rate of wound wounds

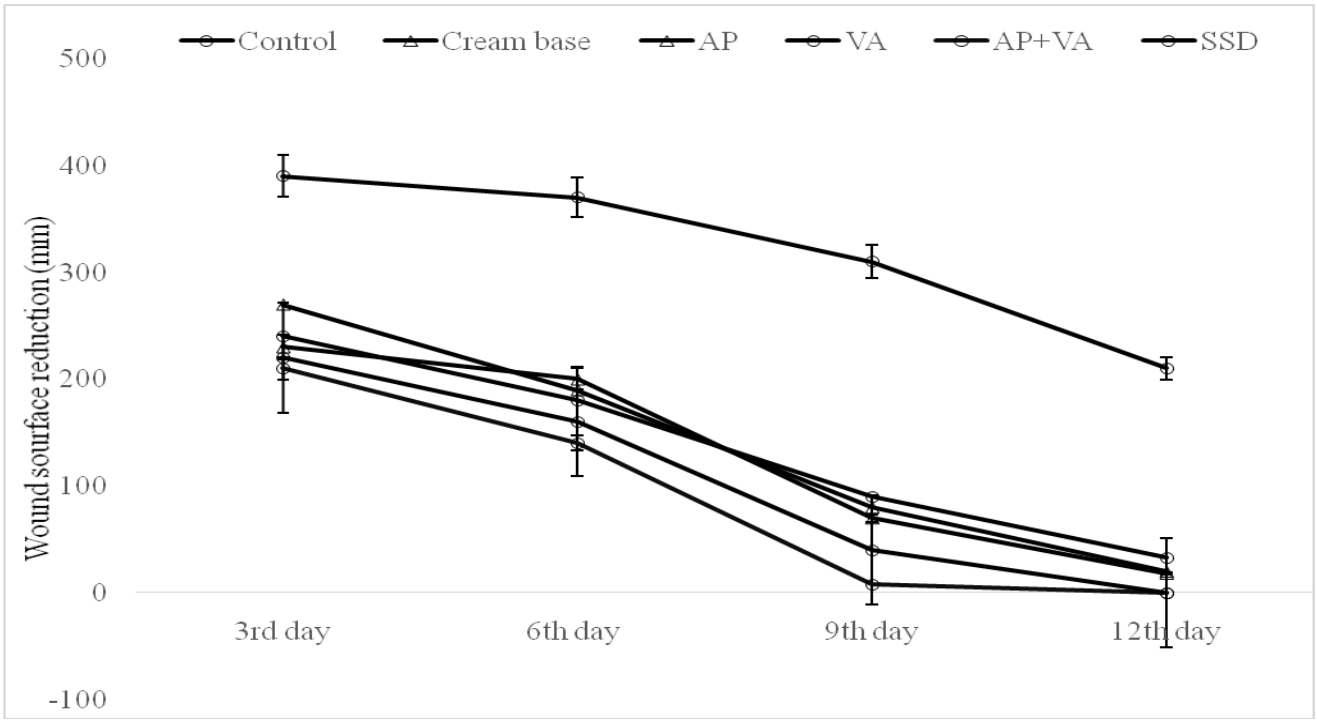

Fig. 2: Wound epithelization in experimental rats

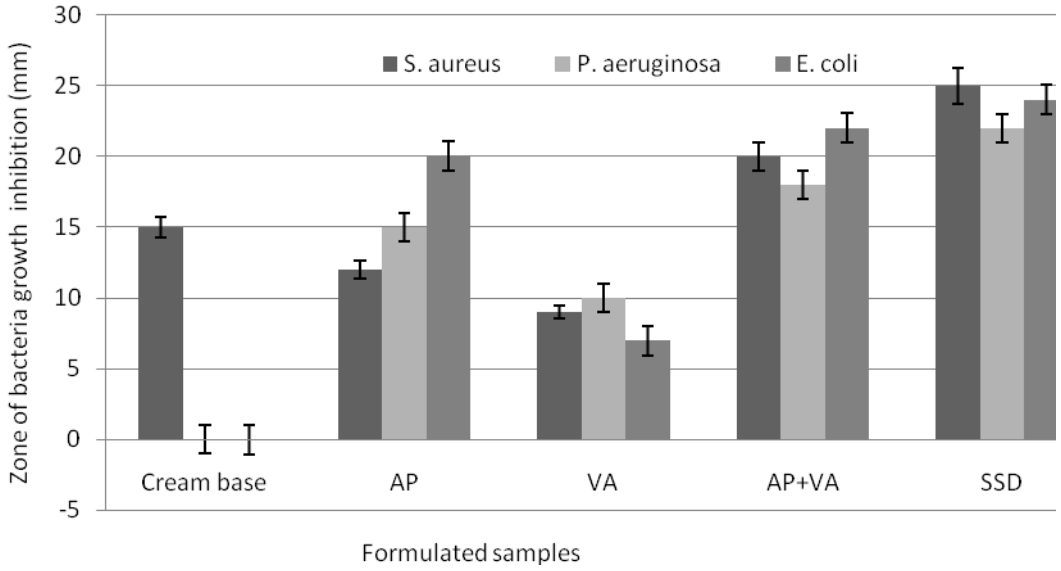

Fig 3: Antibacterial activities of the cream samples against wound susceptible bacteria

DISCUSSION

Methanol extraction: The solvents used for the plant extraction is important as the relative quantities and the type of bioactive fractions extracted depends critically on the type of solvents used. The choice of methanol was based on its reputation as an effective solvents for the extraction of bioactive plant constituent especially for preliminary investigations of activity particularly those with suspected aqueous solubility [24, 31]. Methanol was thus selected because of its high throughput, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate [31, 32]. High extraction throughput and type of bioactive constituents are related to relative solubility of the bioactivities. Methanol has been used for the extraction of the bioactive constituents contained in the leaves and aerial parts of *V. amygdalina* and *A paniculata* respectively because of the predetermined relative high deferential and preferential solubility of certain phenolic and non-phenolic compounds with wound healing potential as determined and reported in previous articles [33,10].

VA and AP both contain a variety of bioactive components some of which have been linked with antioxidant, antimicrobial, analgesic and anti-inflammatory activities which are either directly or indirectly linked to accelerating wound healing. The methanol extract of the leaves of *V. amydalina* and aereal parts of *A. paniculata* were used for the formulation of the shear butter based creams for topical application and investigated for its wound healing potential. *V. amydalina* and *A. paniculata* have individually been evaluated for wound healing activities [10, 33].

Herbal medicine is at the center of various interventions used in several indigenous traditional approaches to healthcare. Herbal products have been shown to accelerate wound healing by several mechanisms which include enhancing blood clotting, disinfect wounds, and accelerated tissue regeneration [34]. In this study, excision wound model was used to establish the healing potentials of the three formulated creams, the polyherbal cream (AV+VA) exhibited the highest wound healing activities which can be related to the presence of the combined phytochemicals which includes flavonoids, saponins, phenols and tannins which are present in AP and VA [10,35-37]. It then will surface to state that some or all of the constituent secondary metabolites in these herbal materials are the bioactive chemical entities that are responsible for the wound-healing activities of these herbal formulations [8].

Formulation of cream and characterization

Cream Formulation: The extracts were formulated as cream to favour the topical transport of extracts in the tissue thereby, increasing the local bioavailability of the extracts. The topical cream will also avoid gastrointestinal intestinal contact and minimize generalized and first pass effects. The W/O based cream, is sure to enhance the solubility and optimal delivery due to enhanced permeation of both the hydrophilic and lipophylic phytochemical constituents of the herbs [38, 39]. Apart from the therapeutic activity of the herbs the water and oil in the cream is to ensure the continuous maintenance of a moist environment through the aqueous and humectants properties of

the aqueous and oil moieties respectively that constitute the emulsified system [25].

Colour: The green colour of the VA, AP and their combinations is typically due to the presence of chlorophyll. The non-change in colour of the formulated creams shows the relative stability of the creams. The cream showed consistent stability on storage at especially normal environmental temperature even when preservative is not part of the formula. The stability may be linked to the presence of antibacterial moieties as component of the phyto constituents. Polyphenols is a major component of the VA and AP and have been identified as one of the main constituents that protect against microbial spoilage due to their antimicrobial activities against a wide variety of bacteria that are responsible for microbial spoilage of food and medicine [40, 41]. The stability of VA, AP and VA+AP creams may so be tracked to the presence of several polyphenolic constituents.

The retention of the soft creamy consistency of the formulations between the temperature of 5 ^{o}C and 27 ^{o}C as well as the melting at 40 ^{o}C indicates the optimum optical temperature range for the physical stability of the creams. The results show that the creams are effectively stable at room temperature (≈ 27 ^{o}C) and must not be stored in environments with high temperature as sure to 40 ^{o}C and above.

The pH of the human skin is typically within the range of 4.5 to 6.0 [41]. The pH of 4.8 and 5 obtained for the shear butter cream base and the herbal creams respectively (Table 3) show that the formulated creams are within the optimal pH range indicated for the skin. The unchanging pH values of creams within the 3 months period in the various temperature environments the effective stability of the products.

Pharmacological evaluation: In the development of drugs used for enhancing wound healing, animal study is an important step before the actual study in human subjects. The use of the Albino Wister rats constitutes an excellent model for the evaluation of the skin wound healing potential of the candidate medicines. The use of rats particularly allows for the standardization of the type, size, shape, and dept of the wounds. The Wister albino rats were selected in this experiment because of its ready availability, low cost, small size and high reliability of the experimental outcomes of results [42].

An effective wound healing agent should accelerate the natural tissue repair by providing a moist dressing/wound interface, absorb or remove excess exudate, provide thermal insulation, prevent contamination, and provide an environment conducive to the body's natural defense mechanisms [43]. The W/O emulsified cream containing the herbal extracts is able to effectively do all of these. The water in oil cream provides a moist environment by the presence of the water molecules in the cream milieu as well as an oily layer that prevents the loss of water and heat from the sourface. The cream forms a film that provides a physical barrier for contaminants as well antimicrobial properties as conferred by the herbal extracts.

Wound epithelialization is one of the parameters used in monitoring the healing of wounds and it occurs when epithelial cells covers the wound bed providing coverage for the new tissue [44]. The animals treated with AV+VA grew fresh hair which covered the healed wounded area on the 12th day, which is a complete epithelization, this accelerated wound healing process is stimulated by different biological events such as fibroplasias, collagen synthesis, and wound contraction [7]. Many mechanisms such as increase rate of epithelization, neovascularization, scavenging of destroying free radicals, inflammation reduction and control of infection due to the presence of antimicrobial, anti-inflammatory and antioxidant constituents found in the formulated herbal creams may be associated with the healing activities of the formulated creams [45].

Epithelialisation is the process by which the epidermal cells are restored after damage due to injury. Wound epithelialization constitutes one of the major intrinsic activities during the wound healing process and also constitutes one of the major parameters used in monitoring progress in wound healing [44].

The rats treated with AV+VA grew fresh hair which covered the healed wounded area on the 12th day. This is an indication of a successful complete epithelization due to the accelerated wound healing process as stimulated by different biological events such as fibroplasias, collagen synthesis, and wound contraction and regeneration [7].

In wound healing process, infection can delay the progress of the process by several mechanisms such as decreasing blood supply, promoting disorder leukocyte function, prolonging inflammatory and debridement phases and producing proteolytic enzymes [46]. Since infection is the complication of wound healing, therefore plants have an extensive potential for the management and treatment of wounds with their antimicrobial, anti-inflammatory and antioxidant activities [47, 48]. This study showed that the polyherbal cream (AP+VA) exhibited the most potent anti-bacterial properties against the three pathogens that are known to commonly infect

wounds. The potent anti-bacterial activity of the polyherbal cream (AP+ VA) may be attributed to the synergistic effect of their phyto-constituents [49, 50]. Both plants are rich in phenolic compounds especially flavonoides which promote the wound-healing process mainly due to their astringent and antimicrobial properties, which seems to be responsible for wound contraction and increased rate of epithelialisation [51, 52].

Anti-inflammatory agents play important role in wound healing. Inflammation is a protective process conducted by the organism with the purpose of removing the harmful stimuli and initiating the operation of healing, the healing process could be delayed by excessive and imbalanced inflammation which can result in tissue or cancer development [53]. The anti-inflammatory activities of *A. paniculata* and *V. amygdalina* have been identified by several studies [54-56].

The antiinflamatory benefits of AP and VA may be linked to tannin component of their phyto-constituents. The tannins are also known to enhance wound healing by precipitating proteins in damaged tissue resulting in scab formation. This leads to decrease tissue edema and exudation along with reducing the permeability of capillaries in the wounded area [57].

Antioxidants promote wound healing by preventing cell damage and death by oxidative stress. Topical application of antioxidant compounds on patients or animals have shown significant improvement in wound healing and protective tissues from oxidative damage [45]. Research has also shown that compounds with free radical scavenging properties could have an important role in survival of ischaemic skin flaps and promotion of wound healing [54]. The antioxidant activities of *A. paniculata* and *V.amydalina* had been demonstrated through the neutralization of excess free radicals and cell protection against their toxic effects [11, 58]. The presence of phenolic compounds in the herbal preparations is likely to be responsible for the free radical scavenging effects observed. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [59].

The wound healing activity of Shear butter (*Vitellaria paradoxa*) may be attributed to their high content of triterpenes. These naturally occurring alcohol and their derivatives have been reported to show anti-inflammatory, antimicrobial and anti-angiogenic activities [60, 61].

Silver sulphadiazine (Dermazin ®) is the contemporary topical pharmaceutical product used for the treatment of wounds due to its antimicrobial efficacy but its long time use is limited by delayed wound healing especially on broad wounds [62, 63].

CONCLUSION

An effective wound healing agent must appropriately reduce the time taken for natural recovery of the wounded tissue, reduce pain, prevent infection as well as be nontoxic, easily available and of low cost. The shear butter cream as well as the mono and poly -herbal creams of the *Androphis paniculata* and *Vernonia amygdalina* were elegant and stable during the ninety days storage time. The wound healing activities of the poly-herbal cream was generally superior to those of the mono-herbal creams of *Androphis paniculata* and *Vernonia amygdalina*, the shear butter cream base. The *Androphis paniculata* and *Vernonia amygdalina* polyherbal cream also showed a faster onset and final wound closure as well as growth of hairs when compared with the standard and prototype contemporary wound healing product: Dermazine®. Nevertheless, the shear butter and the water in oil formulation technique used to produce the poly-herbal cream no doubt contributed to the veracious wound healing activity of the poly-herbal cream.

Conflict of interest statement: We declare that there is no conflict of interest as far as this work is concerned.

REFERENCES

1. Murthy S, Gautam MK, Goel S, Purohit V, Sharma H, Goel RK. Evaluation of *in vivo* wound healing activity of *Bacopa monniera* on different wound model in rats. BioMed Res Inter. 2013; doi.org/10.1155/2013/972028.
2. Percival SL, Thomas JG, Williams DW. Biofilms and bacterial imbalances in chronic wounds: anti-Koch. Int Wound J. 2010; 7: 169-75.
3. Norman R, Matzopoulos R, Groenewald P, Bradshaw D. The high burden of injuries in South Africa. Bull World Health Org. 2006.
4. Adigun IA, Rahman GA, Yusuf IF, Ofoegbu CKF. The point prevalence and cost of wound management in a Nigerian teaching hospital. Nig Med J. 2010; 51(1): 23-5.

5. Gonzalezet AT, Costa TF, Andrade ZA, Medrado AR . Wound healing- A literature review. An Bras Dermatol. 2016; 91(5): 614–20.
6. Shaw T, Martin P. Epigenetic reprogramming during wound healing: loss of polycomb-mediated silencing may enable upregulation of repair genes. EMBO Rep. 2009; 10: 881-6.
7. Daunton C, Kothari S, Smith L, Steele D. A history of materials and practices for wound management. Wound Pract and Res, 2012; 20(4): 174-86.
8. Builders PF, Builders MI. Wound Care: Traditional African Medicine Approach. Chapter contributed to the book topics on Worldwide Wound Healing – Innovation in Natural and Conventional Methods. In Tech, 2016; Chapter 1: 1-26. DOI: 10.5772/62668.
9. Aziz DM, Wsoo MA, Ibrahim BM. Antimicrobial and antioxidant activities of extracts from medicinal plant ginger (*Zingiber officinale*) and identification of components by gas chromatography. Afr J Plant Sci. 2015; 9 (10): 412–20.
10. Al-Bayaty, F H., Abdulla, MA., Hassan, AMI., Ali, HM. Effect of *Andrographis paniculata* leaf extract on wound healing in rats, Nat Prod Res. 2012; 26(5): 423-9.
11. Nayak, BS and Pereira, LM. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. BMC Comp Alt Med. 2006; 6**(**41). Doi:10.1186/1472-6882-6-41.
12. Shivananda NB, Sivachandra RS, Orette FA, Chalapathi RAV. Effects of *Hibiscus rosa sinensis* L (Malvaceae) on wound healing activity: a preclinical study in a Sprague Dawley rat. Int J Low Extrem Wounds. 2007; 6(2): 76-81.
13. Jarukamjorn K, Nemoto N. Pharmacological aspects of *Andrographis paniculata* on Health and its Major Diterpenoid Constituent Andrographolide. J Health Sc. 2008; 54(4): 370-81.
14. Hossain MS, Urbi Z, Sule A, Rahman AH. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. The Sci World J. 2014; doi:10.1155/2014/274905.
15. Akhbar S. *Andrographis paniculata*: A review of pharmacological activities and clinical effects. Alternat Med Effect 2011; 16(1): 66-77.
16. Singha PK, Roy S, Dey S Antimicrobial activity of *Andrographis paniculata*. Fitoterapia 2003; 74: 692-94.
17. Johri RK, Singh C. Medicinal uses of *Vernonia* species. J Med Aromat Plt Sc. 1997; 9: 744-52.
18. Clement E, Osayemwenre E, Imieje V, Ahomafor J, Akunyuli C, Udu- Cosi AA, Onyekaba T, Osakue J, Ali I, Falodun A. Significance of bitter leaf (*Vernonia amagdalina*). In: Tropical diseases and beyond: A Review. Malar Chemoth Cont 3:120. doi:10.4172/2090-2778.1000120
19. Farombi EO, Owoeye O. Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. Int J Environ Res Pub Health 2011; 8: 2533-55.
20. Erasto P, Grierson DS, Afolayan AJ. Evaluation of Antioxidant activity and the fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa. *Food Chem*. 2007; 104: 636-42.
21. Ibrahim TA, Ajala L, Adetuyi, FO, Jude-Ojei B. Assessment of the antibacterial activity of *Vernonia amygdalina* and *Ocimum gratissimum* leaves on selected food borne pathogens. *EJEAFChe.* 2009; 8(11):1212-18.
22. Njan AA, Adzu B, Agaba AG, Byarugaba D, Díaz-Llera S et al. The Analgesic and antiplasmodial activities and toxicology of *Vernonia amygdalina*. J Med Food. 2008; 11: 574-58.
23. Adikwu PC, Amon A, Nambatya G, Adzu B, Imanirampa L et al. Acute toxicity, antipyretic and antinociceptive study of the crude saponin from an edible vegetable: *Vernonia amygdalina* leaf. Int J Biol Chem Sci. 2012; 6: 1019-28.
24. Adesanoye OA, Farombi EO. Hepatoprotective effects of *Vernonia amygdalina* (Astereaceae) in rats treated with carbon tetrachloride. Experimental Toxicol Pathol. 2009; 16: 891-95.
25. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecul.* 2009; 14: 2167-80; doi:10.3390/molecules14062167
26. Builders PF, Kabele-Toge B, Builders M, Chindo BA, Anwunobi PA, Isimi YC. Wound healing potential of formulated extract from *Hibiscus sabdariffa* calyx. Indian J Pharm Sci. 2013; 75(1): 45-52. Doi: 10.4103/0250-474X.113549.
27. Dave V, Kumar D, Lewis S, Paliwal S. Ethosome for enhanced transdermal drug delivery of aceclofenac. Inter J Drug Del. 2010; 2: 81-92.
28. Uno H, Kurata S, Chemical agents and peptides affect hair growth. J Investig Dermatol. 1993; 101: 143S-147S.
29. Bhaskar A, Nithya V. Evaluation of the wound-healing activity of *Hibiscus rosa sinensis* L (Malvaceae) in Wistar albino rats. Indian J Pharmacol. 2012; 44(6): 694-8.
30. Agyare C, Kisseih E, Kyere YI, Ossei PPS. Medicinal plants used in wound care: Assessment of wound healing and antimicrobial properties of *Zanthoxylum leprieurii.* Biol Scs Pharm Res. 2014; 2(8): 081-9.

31. Lalitha G, Nazeema TH, Sharmila L. Phytochemical screening and evaluation of antimicrobial activity, antioxidant activity and fibronolytic activity of leaves of *Andrographis paniculata* (leaf). Int J Pharm Bio Sci. 2015; 6(2): 475-84.
32. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009; 14: 2167-80. doi:10.3390/molecules14062167
33. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju HJ. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. J Food Drugs Anal. 2014; 22: 296-302.
34. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. Arab J Chem. 2017; 10(1): 1193-9.
35. Eyo JE, Uzoibiam BO, Ogbanya KC, Nnaji TO. Comparative evaluation of wound healing effects of *Ocimum gratissimum*, *Vernonia amydaline* and *Zingiber offinalis* extract on incision wound model in rats. Pharmacolonline 2014; 3: 44-50.
36. Syed NA, Hasan TN, Aalam SMM. Evaluation of wound healing potential of C*hicorium Intybus.* L (Asteraceae) in rats. Iran J Pharmacol Therap. 2008**;** 7(2): 181-4.
37. Vringer T, Ronde HA. Preparation and structure of a water-in-oil cream containing lipid nanoparticles. J Pharm Sc.1995; 84(4): 466-72.
38. Nacu JA. Behavior of soybean oil-in-water emulsion stabilized by nonionic surfactant. J Coll Interface Sci. 2003; 259: 374-81.
39. Karou D, Savadogo A, Canin A, Yameogo S, Montesano C, Simpore J, Colizzi V, Traore AS. Antibacterial activity of alkaloids from *Sida acuta*. Afric J Biotech. 2005; 4 (12): 1452-7.
40. Waqas MK, Akhtar N, Ahmad M, Murtaza G, Khan HM, Iqbal M, Rasul A, Bhatti NS. Formulation and characterization of a cream containing extract of fenugreek seeds. Acta Pol Pharm. 2010; 67(2):173-8.
41. Martin DWA, Wysocki AB. Rat Models of Skin Wound Healing. In: Conn P.M. (eds) 2008; Sourcebook of Models for Biomedical Research. Humana Press.
42. Wijetunge DB. Management of acute and traumatic wounds: main aspects of care in adults and children. Am J Surg. 1994; 167: 56S-60S.
43. Eming SA., Martin P, Tomic-Canic M. Wound repair and regeneration: Mechanisms, signaling, and translation. Sci Transl Med. 2014; 6(265): doi:10.1126/scitranslmed.3009337.
44. Kahkeshani N, Farahanikia B, Mahdaviani P, Abdolghaffari A, Hassanzadeh G, Abdollahi M, et al. Antioxidant and burn healing potential of Galium odoratum extracts. Res Pharm Sci. 2013; 8(3): 197–203.
45. Kumari P, Yadav P, Verma PR, Kumar S, Arya A. A review on wound healing properties of Indian medicinal plants. Ind J Fund Appl Life Sci. 2013; 3(1): 220-32.
46. Annan K, Houghton PJ. Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of Ficus asperifolia Miq. and Gossypium arboreum L., wound-healing plants of Ghana. J Ethnopharmacol. 2008; 119(1):141-4.
47. Rajalakshmi V, Cathrine L. Phytochemical screening and antimicrobial activity of ethanolic extract of Andrographis paniculata. J Pharmacog Phytochem 2016; 5(2): 175-7.
48. Odunbaku OA, Ashidi JS. Antimicrobial effect of ethanol leaf extracts of selected medicinal plants on some human pathogenic microbes. Inter J Sc Advanc Technol. 2012; 2(8): 19-22.
49. Oberyszyn TM. Inflammation and wound healing. Front Biosci. 2007; 12(8-12): 2993–9.
50. Kahkeshani N, Farahanikia B, Mahdaviani P, Abdolghaffari A, Hassanzadeh G, Abdollahi M, et al. Antioxidant and burn healing potential of *Galium odoratum* extracts. Res Pharm Sci. 2013; 8: 197-203.
51. Rajeshkumar S. Antimicrobial effect of King of bitter Andrographis paniculata and traditional herb Aegle marmelos against clinical pathogens. Int J PharmTech Res. 2014-2015; 7(2): 325-9.
52. Kigigha LT, Onyema E. Antibacterial activity of bitter leaf (Vernonia amygdalina) soup on Staphylococcus aureus and Escherichia coli). Sky J Micro Res. 2015; 3(4): 041-5.
53. Dwivedi D, Thanwar M, Gharia AK. A phytochemical investigation on Andrographis paniculata. J. Chem. Pharm. Res. 2015; 7(10): 822-7.
54. Sheeja K, Shihab PK, Kuttan G. Antioxidant and anti-inflammatory activities of the plant Andrographis paniculata Nees. Immunopharmacol Immunotoxicol. 2006; 28(1): 129-40.
55. Georgewill OA, Georgewill UO. Evaluation of the anti-inflammatory activity of extract of *Vernonia Amygdalina.* Asian Pac J Trop Med. 3(2); 2010: 150-1.
56. Sarwar M, Attitala IH, Abdollahi M. A review of recent advances in pharmacological studies on medicinal plants , animal studies are done but clinical studies need completing, Asian J Anim Vet Advanc. 2011; 6(8): 867-83.

57. Mendonca FA, Passarini Junior JR, Esquisatto MA, Mendonca JS, Franchini CC, Santos GM. Effects of the application of Aloe vera (L.) and microcurrent on the healing of wounds surgically induced in Wistar rats. Acta Cir Bras. 2009; 24(2):150-5.
58. Thang PT, Patrick S, Teik LS, Yung CS. Anti-oxidant effects of the extracts from the leaves of Chromolaena odorata on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine-xanthine oxidase induced damage. Burns. 2001; 27(4): 319-27.
59. Sheeja K, Shihab PK, Kuttan G. Antioxidant and anti-inflammatory activities of the plant *Andrographis Paniculata* Nees. Immunopharmacol Immunotoxicol. 2006; 28(1):129-40.
60. Farombi EO, Owoeye O. Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. Int J Environ Res Pub Health. 2011; 8(6): 2533-55. doi: 10.3390/ijerph8062533.
61. Builders MI, Alemika T, Aguiyi JC. Antimalarial activity and isolation of phenolic compounds from *Parkia biglobosa*, *IOSR* J. Pharm Biol Sc. 2014; 9: 78-85.
62. Akihisa et al. Triacylglycerol and triterpene ester composition of shea nuts from seven African countries. J Oleo Sci. 2011; 60(8): 385-91.
63. Maranz S et al. Nutritional values and indigenous preferences for shea fruits (Vitalleria paradoxa Gaertn . F.) in African agro-forestry parklands. Econ Bot. 2011; 58: 588-600.
64. Ezzati A, Bayat M, Taheri S, Mohsenifar Z. Low-level laser therapy with pulsed infrared laser accelerates third-degree burn healing process in rats. J Rehabi Res Dev/ 2009; 46(4): 543-54.
65. Upadhyay NK, Kumar R, Siddiqui MS, Gupta A. Mechanism of wound healing activities of *Hippophae rhamnoides* L leaf extract in experimental burns. Evid Base Complit Alternat Med. 2011. doi:10.1093/ecam/nep189.